

Disease Reaction of Diverse Sources of *Lycopersicon* to *Xanthomonas campestris* pv. *vesicatoria* Pepper Strain Race 2

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ABSTRACT

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More than 4,000 named varieties and *Lycopersicon* plant introduction accessions were evaluated for disease reaction to one isolate of *Xanthomonas campestris* pv. *vesicatoria*, causal agent of bacterial spot of tomato. None of the accessions displayed a symptomless reaction to spray inoculation, but differences in symptom severity were observed among genotypes. Several lines with high levels of resistance were identified.

Lycopersicon spp., but resistance (expressed as absence of symptoms) has not been found (4,5,13). This paper reports results of screening the plant introduction (PI) collection of *Lycopersicon* to one isolate of *X. vesicatoria*.

MATERIALS AND METHODS

Plant material and growing conditions. All PI accessions available for distribution (4,424) were obtained from the North

Xanthomonas campestris pv. *vesicatoria* (Doidge) Dowson (*X. vesicatoria*) causes bacterial spot of pepper and tomato. Disease development is favored by plant wounding, high air temperatures, rain, and wind (3,12). Control measures are crop rotation, seed treatments, and foliage sprays. Severe plant infection may reduce fruit yield and quality. Genetic resistance to *X. vesicatoria* has been reported with *Capsicum* (10,11). Levels of susceptibility have been identified in

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Table 1. Reaction of 16 *Lycopersicon* spp. to *Xanthomonas campestris* pv. *vesicatoria* pepper strain race 2

Population	Lines tested	Mean score	Mean DI
Verified gene stocks	63	2.7 ^a	101 ^b
Tetraploids	15	2.4	114
Auto-diploids	4	2.2	88
Male steriles	23	2.3	100
<i>L. glandulosum</i>	10	2.4	88
<i>L. hirsutum</i>	15	2.9	94
<i>L. hirsutum</i> f. <i>glabratum</i>	7	2.5	88
<i>L. peruvianum</i>	79	2.6	96
<i>L. peruvianum</i> var. <i>humifusum</i>	3	2.7	106
<i>L. cheesmanii</i> f. <i>minor</i>	4	3.3	107
<i>L. pimpinellifolium</i>	208	2.6	94
<i>L. esculentum</i> × <i>L. hirsutum</i>	4	2.9	94
<i>L. esculentum</i> × <i>L. peruvianum</i>	4	2.1	89
<i>L. esculentum</i> × <i>L. pimpinellifolium</i>	2	3.0	97
<i>L. esculentum</i> × <i>L. pimpinellifolium</i> (suspected)	157	2.7	90
<i>L. esculentum</i>	3,826	2.7	93

^a Weighted mean: 0 = no disease symptoms, 1 = 1-3% leaf necrosis, 2 = 3-6% leaf necrosis, 3 = 6-12% leaf necrosis, 4 = greater than 12% leaf necrosis.

^b Weighted mean of standardized disease index (DI); Chico III = 100.

Central Regional Plant Introduction Station, Ames, IA 50011. Eighty-six percent of the collection was composed of *L. esculentum* accessions. The remaining 14% included various genetic stocks, members of *L. glandulosum*; *L. hirsutum*, including f. *glabratum*; *L. peruvianum*, including var. *dentatum* and *humifusum*; *L. cheesmanii* f. *minor*; *L. pimpinellifolium*; *L. esculentum*, including f. *pyriforme* and var. *cerasiforme*; and various known or suspected species crosses (9). Seed of *L. esculentum* cv. Chico III (susceptible check) was obtained from A. L. Castle, Inc., Morgan Hill, CA 95037.

Seed of each line was sown in wooden flats containing equal parts of loam, peat, and perlite. Each flat consisted of five rows divided in half to produce 10 four-hill plots. The susceptible check and 9 PI lines were included per flat. Hills were thinned to one plant when the first true leaf emerged. Plants were grown on greenhouse benches until they reached the second or third true-leaf stage. At this

time, they were placed in an inoculation chamber, which consisted of a portion of the greenhouse bench equipped with misters and enclosed with Monsanto 602 plastic.

Pathogen culture. A local isolate of *X. vesicatoria* was obtained from infected tomato fruit collected at Gilbert, IA, 10 mi. north of the Ames Plant Introduction farm. Isolate identity was confirmed by comparing morphological and biochemical characteristics with *X. vesicatoria* (ATCC 11551), obtained from the American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852. Both isolates produced a negative gram stain and a weak cytochrome oxidase reaction and were pathogenic on Chico III. Spray inoculation of the Iowa isolate on Chico III, *Capsicum annuum* cv. Early Calwonder, and *Solanum melongena* cv. Blackbell resulted in development of necrotic lesions on the foliage. The *Capsicum* accession PI 163192 displayed a symptomless reaction to the Iowa isolate with spray-inoculation

and a hypersensitive reaction after infiltration-inoculation of its leaves (8). In contrast, similar spray inoculations with ATCC 11551 produced symptoms only on Chico III tomato plants. Pathogenic differences among isolates of this bacterium have been identified by Cook and Stall (2). According to their classification, ATCC 11551 is a tomato strain and the Iowa isolate is a pepper strain (race 2) of the bacterium.

Cultures of *X. vesicatoria* lost virulence when maintained on Difco nutrient agar for more than a few weeks. For this reason, the pathogen was maintained in Chico III host plants and reisolated from foliage lesions whenever needed.

Inoculation. *X. vesicatoria* was increased on Difco nutrient agar at 25 C. Inoculum was prepared by gently scraping the culture material from 24-hr-old plates and suspending the bacteria in sterile distilled water. A Bausch & Lomb Spectronic 20 set at 540 nm was used to adjust the final inoculum concentration to provide an absorbance reading of 0.10. Suspensions with this optical density contained about 10⁸ colony-forming units per milliliter. Inoculations were made during the summer when chamber temperatures averaged 25 ± 2 C. Relative humidity was maintained at 95–98% inside the chamber. Plants were incubated in the chamber 24 hr before and 48 hr after inoculation. Inoculum was applied with a DeVilbiss atomizer connected to a compressed-air line set at 85 psi. The atomizer was held 4–6 in. above the plant canopy and moved so that all foliage was finely wetted.

Disease evaluation. Incidence and severity of disease symptoms were recorded 10–14 days after inoculation. The top two fully expanded leaves of each plant were used to make the following severity ratings: 0 = no disease lesions, 1 = 1–3% of leaf area necrotic, 2 = 3–6% of leaf area necrotic, 3 = 6–12% of leaf area necrotic, and 4 = greater than 12% of leaf area necrotic. These disease ratings were used to obtain a mean disease rating along with its standard deviation and a standardized disease index (DI). DIs were computed by dividing the mean disease rating of each line in a flat by the mean disease rating of susceptible Chico III grown in the same flat and multiplying by 100. The DI became a weighted mean when the line was grown in more than one flat.

RESULTS AND DISCUSSION

None of the lines displayed a symptomless reaction to spray inoculation with the Iowa isolate of *X. vesicatoria* in these studies. This was unexpected because genes for resistance have been found within the *Capsicum* species (1). If present, this resistant genotype possibly 1) was hidden in a heterogeneous line, 2) developed atypical symptoms resulting in

Table 2. Reaction of 4,424 *Lycopersicon* accessions by source to *Xanthomonas campestris* pv. *vesicatoria* pepper strain race 2

Source	Lines tested	Mean score	Mean DI	Source	Lines tested	Mean score	Mean DI
Afghanistan	13	2.6 ^a	90 ^b	Italy	62	2.5	120
Argentina	67	2.9	89	Japan	5	2.9	100
Australia	28	2.7	88	Kenya	1	3.8	115
Balearics	1	1.8	78	Lebanon	2	2.6	86
Baluchistan	3	2.4	83	Malawi	2	3.8	116
Bolivia	78	3.0	91	Malaysia	6	2.2	85
Brazil	104	2.7	88	Manchuria	13	2.5	92
British Guiana	1	3.3	93	Mexico	103	2.4	100
Bulgaria	30	2.7	91	Morocco	16	2.7	95
Canada	120	3.1	105	Nepal	1	3.0	90
Canary Islands	1	2.8	79	Netherlands	23	2.7	96
Ceylon	1	2.0	160	New Caledonia	1	2.0	62
Chile	59	2.2	95	New Guinea	1	2.4	85
China	51	2.5	85	New Zealand	1	3.3	93
China, PRC	334	2.6	94	Nicaragua	31	3.2	100
China, Taiwan	10	3.4	107	Nigeria	11	2.5	91
Colombia	87	2.9	90	Norway	1	3.0	120
Cook Islands	2	3.0	93	Palestine	2	2.5	87
Costa Rica	46	3.0	95	Panama	40	2.8	90
Cuba	6	3.0	85	Peru	420	2.5	94
Czechoslovakia	65	3.0	104	Philippines	12	2.7	84
East Africa	1	3.3	108	Poland	45	2.9	91
Ecuador	141	2.9	89	Puerto Rico	22	2.6	88
Egypt	2	2.5	93	Romania	5	2.9	103
El Salvador	421	2.7	104	Scotland	3	2.6	92
England	12	1.9	62	South Africa	17	2.6	90
Ethiopia	17	2.5	92	South America	1	4.0	133
France	17	2.5	90	Spain	10	2.2	93
French Guiana	13	3.2	86	Sweden	5	2.8	96
Germany	17	2.7	86	Switzerland	1	2.5	125
Ghana	10	2.6	97	Syria	6	2.6	87
Great Britain	14	3.6	107	Tasmania	1	2.0	73
Greece	5	2.4	83	Thailand	5	2.8	104
Guadeloupe	14	3.1	89	Turkey	195	2.9	100
Guatemala	216	2.5	93	Uruguay	1	2.3	85
Honduras	94	2.9	97	USA	642	2.8	97
Hungary	134	3.0	95	USSR	91	2.7	97
India	81	2.5	100	Venezuela	26	2.2	85
Iran	63	2.7	91	West Pakistan	3	2.9	85
Iraq	2	2.4	93	Yugoslavia	159	3.3	99
Israel	15	2.6	96	Zaire	1	3.3	83

^a Weighted mean: 0 = no disease symptoms, 1 = 1–3% leaf necrosis, 2 = 3–6% leaf necrosis, 3 = 6–12% leaf necrosis, 4 = greater than 12% leaf necrosis.

^b Weighted mean of standardized disease index (DI): Chico III = 100.

Table 3. *Lycopersicon* accessions and selected commercial cultivars least susceptible to *Xanthomonas campestris* pv. *vesicatoria* pepper strain race 2 (Iowa isolate)

PI code	Source	Mean rating	Mean DI
127808	Peru	1.0 ± 0.0 ^a	33 ^b
390687	Peru	1.0 ± 0.0	35
379054	Ecuador	1.3 ± 1.4	36
375937	United States	1.3 ± 0.5	41
Roma VF	United States	1.0 ± 0.8	44
Rutgers	United States	1.0 ± 0.8	44
273085	El Salvador	1.4 ± 1.3	46
155368	Peru	1.5 ± 1.0	46
379050	Ecuador	1.7 ± 0.8	46
129019	Peru	1.4 ± 1.1	47
117898	Brazil	1.4 ± 1.3	48
231730	United States	1.7 ± 0.6	48
117899	Brazil	1.3 ± 1.0	49
126426	Peru	1.3 ± 0.5	50
128215	Bolivia	1.8 ± 1.0	50
127807	Peru	1.5 ± 1.0	50
283930	Czechoslovakia	1.5 ± 1.3	50
155378	Peru	1.5 ± 0.6	50
128660	Peru	1.5 ± 0.6	50
195784	Guatemala	1.5 ± 0.6	50
272626	El Salvador	1.3 ± 0.5	50
C-28	United States	1.0 ± 0.5	55
Manalucie	United States	2.0 ± 1.0	62
Tropic	United States	1.8 ± 0.5	64

^a Weighted mean: 0 = no disease symptoms, 1 = 1-3% leaf necrosis, 2 = 3-6% leaf necrosis, 3 = 6-12% leaf necrosis, 4 = greater than 12% leaf necrosis.

^b Weighted mean of standardized disease index (DI): Chico III = 100.

a susceptible reaction, or 3) may have been undetectable using only one isolate of *X. vesicatoria*, which possibly had several genes for pathogenicity. In general, resistance expressed as a symptomless reaction or hypersensitivity to *X. vesicatoria* is not widespread among *Lycopersicon*.

The mean DI of all lines tested was 93. The mean DI of the susceptible check was 100. There was little difference in susceptibility among cultivars from the same species or source (Tables 1 and 2). Accessions originating in England were possibly less susceptible, with a mean DI of 62. Individual DIs ranged from 33 to 400. Twenty-one lines received DIs of 50 or less, and 565 lines received DIs of 75 or less (Table 3).

L. esculentum accession PI 114490 was often used as a resistant check. This accession produced a mean DI of 61 on

the basis of 1,423 plant evaluations, but individual DIs ranged from 0 to 143. Expression of susceptibility seemed to be influenced by small environmental differences during inoculation and incubation. Because of variations in the greenhouse environment, it was not possible to provide identical conditions for each screening group, making it difficult to identify or quantitate levels of susceptibility consistently.

Several lines with considerable resistance were identified; however, continued work with intermediate levels of resistance should be undertaken in chambers where rigid control of environmental conditions may be achieved. Ultimately, field evaluations under natural epidemics will be needed to determine whether the observed level of intermediate resistance is great enough to be of value or whether attempts should be

made to transfer hypersensitivity genes from pepper. Although intermediate levels of resistance are difficult to incorporate because of their polygenic nature, polygenic pathogen control may be desirable inasmuch as the mutation rate for the pathotype has been found to be relatively high (6,7). Refined techniques could make it possible to transfer and intensify the bacterial spot resistance observed into adapted commercial cultivars.

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